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ORIGINAL PAPER

Investigation of salinity impacts on germination and growth of two forest tree species at seedling stage

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Abstract: Soil salinity is becoming an increasingly serious constraint to plant growth in many parts of the world; this is particularly common in semi-arid and arid zones. This study was conducted to evaluate the effect of different concentrations of salt on seed germination and seedling growth of *Acacia albida* and *A. salicina*. Collected seeds were treated with H_2SO_4 98% for 35 min then left to germinate in a controlled growth chamber. Seeds were grown at salinity levels of 0.1, 0.2 and 0.3 mol·L^{-1} of $NaH_2PO_4.H_2O$. Germination parameters and seedling growth indices were measured after 35 days. The mean of total length of *A. salicina* in all salinity concentrations was more than that of *A. albida*. The fresh weight of stem and root of *A. albida* was more than that of *A. salicina.* Growth of control seedlings was higher than for other treatments and the fresh weight of stems of two species at salt concentrations of 0.2 and 0.3 mol·L^{-1} decreased with increasing concentration of salt. Most control seeds germinated at salinity of 0.1 mol·L^{-1} and germination index, final germination and seeds stamina was greater at low levels of salinity.

Keywords: soil salinity; germination; salt concentration; *Acacia albida*, *Acacia salicina*.

Introduction

Studies of environmental stresses on trees at different stages of development are important for understanding regeneration and successful establishment of plantations. Seed germination, a critical stage in plant life, is most vulnerable to such stresses (Freeman 1973; Catalan et al. 1994). For many plant species, soil salinity is known to reduce growth and development through osmotic stress, ion toxicity, mineral deficiencies and induced physiological and biochemical disorders in metabolic processes (Sohail et al. 2009). Germination and seedling characteristics are the most practical criteria for selecting salt tolerance in plants (Jamil et al. 2006). In recent years, interest in woody legumes has increased widely in view of their potential utilization in revegetation strategies for arid and semi arid areas. The flowering plant family Leguminosae is one of the most important groups of economic plants. It includes three subfamilies: Caesalpinioideae, Mimosoideae and Papilionoideae. Within Mimosoideae, the species *Acacia* is the second largest species in the family Leguminosae (Khelladi et al. 2010). The geographical distribution of *Acacia* species includes 1010 species in Australia, 60 in Asia, 185 in the Americas and 150 in Africa (Bouchenak-Khelladi. et al. 2010). The genus is exploited in natural habitats and plantations for many purposes (Brockwell et al. 2005). Unlike saltbushes, many species of *Acacia* are adapted to sandy habitats and, therefore, are used in sand dune fixation (Giffard, 1964) and are also effective in checking soil erosion (Vengadesan et al. 2002). *Acacia* is an evergreen tree and occurs in almost all habitat types (Bouhle et al. 2008).

Acacia albida Del. is a monotypic genus in the legume subfamily *Mimosoidae*. *A. albida* is a deciduous tree normally growing to 15 m and can reach 25 m or more in southern Africa with a large, rounded crown and spreading branches and trunk diameters of 1 m or more. *A. albida* presents great genotypic and phenotypic diversity (Gassama et al. 2003), and is widespread in semi-arid Africa on a wide range of soil types and in different climates (Simute et al. 1998). Furthermore, because many *Acacia* populations suffer high levels of mortality due to water stress, we tested whether trees in high mortality populations had diminished effects on plant species and soil quality under their canopies (Munzbergova and Ward 2002). *A. salicina* (willow wattle) is a leguminous shrub or small tree introduced to many regions as a multipurpose species. This is an Australian tree species that has been used extensively for forestation in arid areas of the

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Mediterranean basin because it provides high quality wood and fodder and is highly resistant to drought and salinity (Jeddi et al. 2009). Yokota (2003) investigated the effects of salinity on the seedlings of five *Acacia* species, finding that the dry weights of whole plant of *A. ampliceps* and *A. holosericea* decreased markedly under the 2.5% (w/v) and 1.5% NaCl stress conditions. Madsen and Mulliga (2006) showed that *A. salicina* was found to be superior to *Eucalyptus* spp. in its ability to emerge and survive under saline conditions. It was the only species to have seedlings emerge and survive at 0.2 $mol \cdot L^{-1}$ NaCl (20 $dS \cdot m^{-1}$) and the effect of salt on decreasing seedling dry weight was less pronounced for *A. salicina* than for any of the *Eucalyptus* species. *A. salicina* performed significantly better at 300 and 400 mM NaCl than most of the studied eucalypts.

Sarwar Khan et al. (2009) investigated the effect of salinity on growth of forest tree species. Growth parameters decreased as salinity increased. *Acacia* species were reported as salt tolerant. Maximum shoot length and root length was recorded from *A. nilotica* against *A. ampliceps* at same level of salinity (18 $dS \cdot m^{-1}$ ECe). Rehman et al. (2000) reported that significant positive relationships between salinity tolerance I_{50} and the concentration of NaCl are required to reduce final germination to 50% of the control value in DW (dry weight) and FG (Final germination percentage), rate, Ca_{unt}/K_{unt} and Ca_{NaCl}/K_{NaCl}. Some *Acacia* species were screened for their salt tolerance during seed germination in the presence of NaCl. Imbibitions by seeds incubated on NaCl solutions were reduced by only approximately 1% for each 100 $mol \cdot m^{-3}$ increase in NaCl concentration. The adverse effects of NaCl on seed germination, observed in this study appear to result from internal osmotic or ion toxicity effects rather than from restriction of imbibitions (Rehman et al. 1997). The objective of this study was to evaluate the effects of different concentration of salinity on germination and young seedling growth of *A. albida* and *A. salicina*.

Materials and methods

Laboratory experiments were conducted at the Forestry Department of the Natural Resources Faculty, Sari, Iran. The seeds of *A. albida* and *A. salicina* were collected from Natural Resource Bandar Abbas and Research Institute of Forests and Rangelands Chalus. Seeds were scarified with H_2SO_4 98% for 35 min. The seeds were treated, surface sterilized by dipped in 0.1% $HgCl_2$ solution for 15 min, before they were rinsed in sterile distilled water.

Seeds were planted in sterilized glass bottles with solutions of salts at concentrations of 0.1, 0.2 and 0.3 $mol \cdot L^{-1}$of $NaH_2PO_4.H_2O$. Controls were planted in B_5 medium. Glass bottles were maintained in a growth chamber at 25°C with a 16 h photoperiod. Seed germination in each glass bottle was observed weekly and germinated seeds were counted. Final germination percentage (F_G) (%) and mean daily germination (M_{DG}) (% per day) were calculated as:

$$F_G = n / N \times 100 \tag{1}$$

$$M_{DG} = F_G / D \tag{2}$$

where, n is the number of germinated seeds (%), N is the total number of seeds (%), F_G is the final germination percentage (%), D is the number of days to final germination percentage (day). To assess the germination rate (G_R, % per day), the mean germination time was calculated as:

$$G_R = \sum_{i=1}^{n} \frac{n_i}{t_i} \tag{3}$$

where, n_i is the number of germinated seeds on day t_i. Germination index (G_I), S_c is seedling vitality (succulence, mg), and seed stamina (S, mm) were calculated as:

$$G_I = \frac{\sum tn}{N} \tag{4}$$

$$S_c = \frac{F_W \times D_W}{F_W} \tag{5}$$

$$S = \frac{L \times F_G}{100} \tag{6}$$

where, S is the seed stamina (mm), S_c is the succulence (mg), F_W is the seedling fresh weight, D_W is the seedling dry weight (mg) and L is the mean seedling length (mm).

A randomized complete design was used with three replications and 10 seeds or seedlings in each replicate. Data were statistically analyzed using the GLM procedure in the SPSS program. Duncan's test at α = 0.05 and 0.01 was used to compare means within and between treatments.

Results

The effects of salinity on germination and biomass production of *A. albida* and *A. salicina* are shown in Table 1. Seed stamina, stem length, root length, total length of seedlings, as well as germination parameters were significantly affected by species (p <0.01). Moreover, total fresh weight, fresh weight of stem, stem length, total length and fresh weight of root were significantly affected by the interaction betwe en salt concentration (p <0.01) and species (p <0.05). All biomass production and germination parameters of two species were significantly affected by salinity (Table 1). There were no significant differences between species in germination parameters at different salt concentrations ($NaH_2PO_4H_20$), (Table 2). Nevertheless, the impact of salt concentration on these parameters was statistically significant (Table 1).

Table 1. Analysis of variance showing the effect of the species type, different concentration of salinity and their interaction on germination and biomass of *Acacia salicina* Lindl. and *Acacia albida* Dal.

Index	Species			Salt concentration ($mol \cdot L^{-1}$)			Species × Salt concentration ($mol \cdot L^{-1}$)		
	Sum of Square	Mean Square	*F* value	Sum of Square	Mean Square	*F* value	Sum of Square	Mean Square	*F* value
Biomass product									
Stem length(SL, mm)	17.2	2.52	6.8^{*}	149.8	49.9	19.6^{**}	30.7	10.2	4^{*}
Root length(RL, mm)	3.6	0.063	5.7^{*}	32.3	10.7	17.2^{**}	4.5	1.5	2.4^{ns}
Total length(TL, mm)	36.7	4.82	7.6^{*}	316.5	105.5	21.8^{**}	56.6	18.8	3.9^{*}
Fresh weight of stem(FWS, mg)	3800	974.35	3.9^{ns}	102972	34324	35.8^{**}	28178	939	9.8^{**}
Fresh weight of root (FWR, mg)	12.04	120.4	0.1^{ns}	1185	395.2	5.3^{**}	728.1	242.7	3.3^{*}
Dry weight of stem(DWS, mg)	45.3	30.2	1.5^{ns}	1514.4	504.8	17.4^{**}	269.4	89.8	3.1^{ns}
Dry weight of root(DWR, mg)	9.3	4.42	2.1^{ns}	83.4	27.8	6.4^{**}	19.4	6.4	1.5^{ns}
Total fresh of weight (TFW, mg)	424	124.7	3.4^{ns}	12623	42077	34.5^{**}	37462	124	10.2^{**}
Total dry weight(TDW, mg)	96	45.71	2.1^{ns}	2272.5	757.5	16.7^{**}	417.3	139.1	3^{ns}
Succulence (SU, mg)	477	318	1.5^{ns}	31621.1	10540	34.5^{**}	1130	367.8	1.2^{ns}
Germination									
Germination index (GI, % per day)	0.3	0.5	0.6^{ns}	64.5	21.5	38.7^{**}	2.2	0.7	1.3^{ns}
Final germination(FG, %)	66.6	133.2	0.5^{ns}	28700	9566	520.8^{**}	433.3	144.4	0.7^{ns}
Mean daily germination (MDG, % per day)	0.15	0.5	0.3^{ns}	65	21.6	52.1^{**}	0.9	0.3	0.7^{ns}
Germination rate (GR, % per day)	9.7	9.7	0.2^{ns}	1818.7	606.2	14.4^{**}	98.4	32.8	0.7^{ns}
Seed stamina(S, mm)	3544	3544	6.1^{*}	25053.9	8351	14.5^{**}	3908	130	2.2^{ns}

Notes: * and ** mean significant in probability level of 5%, 1%, respectively; n sis not significant.

Table 2. Effect of the interaction between different concentrations of salinity and species type on germination

Index	*A. albida*				*A. salicina*			
	Control	0.1 $mol \cdot L^{-1}$	0.2 $mol \cdot L^{-1}$	0.3 $mol \cdot L^{-1}$	Control	0.1 $mol \cdot L^{-1}$	0.2 $mol \cdot L^{-1}$	0.3 $mol \cdot L^{-1}$
Final germination (FG, %)	83.33^{a}	56.67^{a}	20^{a}	0^{a}	86.67^{a}	73.33^{a}	13.33^{a}	0^{a}
Germination rate (GR, %)	2.09^{a}	1.26^{a}	3.63^{a}	0^{a}	1.96^{a}	2.08^{a}	1.84^{a}	0^{a}
Mean daily germination (MDG, %)	3.97^{a}	2.7^{a}	$.95^{a}$	0^{a}	4.13^{a}	3.49^{a}	$.63^{a}$	0^{a}
Germination index (GI, %)	3.7^{a}	3.3^{a}	1.8^{a}	0^{a}	5^{a}	3.2^{a}	1.6^{a}	0^{a}
Seed stamina (S, mm)	5.59^{a}	1.64^{a}	2.17^{a}	0^{a}	9.89^{a}	7.23^{a}	0.56^{a}	0^{a}

Notes: Means followed by different lower-case letters within columns are significantly different (p<0.01).

The impact on mean fresh weight of roots of interaction between salt concentrations ($NaH_2PO_4.H_20$) and species type in *A. albida* species and control treatment was the highest and there were significant differences with other treatments and other treatment were placed in a similar means range. Stem length in *A. salicina* and control seedlings was longer than for other treatments of interaction salt concentration and species. Mean total length of *A. salicina* seedlings at all salinity concentrations was greater than for *A. albida*. Maximum total length was recorded for control treatment of *A. salicina* seedlings. The total lengths of two species at 0.2 and 0.3 $mol \cdot L^{-1}$ salt concentrations were similar, while total length of *A. salicina* at salinity of 0.1 $mol \cdot L^{-1}$was greater than that of *A. albida*. The fresh weight of stems in *A. albida* and control treatments was greatest and the fresh weight of stems of the two species at 0.2 $mol \cdot L^{-1}$and 0.3 $mol \cdot L^{-1}$ salt concentrations were similar. Maximum total length was recorded for *A. salicina* but fresh weight of stems of *A. salicina* was lower than that for *A. albida*. The total fresh weight and fresh weight of stems was similar for the two species and decreased with increasing salinity concentration in all treatments, but the weight of stems and roots of *A. albida* was more than that for *A. salicina*, and the values of these parameters at all salinities exceeded the fresh weight of roots (Table 3). Greatest final germination was recorded for the control treatment of salt concentration for *A. salicina* and *A. albida*. This parameter decreased as the salinity level of the medium increased and the final germination parameter was a diminishing process. Germination rates for control treatment and 0.1 $mol \cdot L^{-1}$salt concentration were similar for *A. salicina* and *A. albida*.

Germination rate was higher at salinity of 0.2 mol·L^{-1} was higher than at 0.1 mol·L^{-1} for *A. albida* and also higher at 0.1 mol·L^{-1} than that for control treatment of *A. salicina*. However, there was no germination rate because no germination was recorded at 0.3 mol·L^{-1} salt concentration (Table 2). Mean daily germination differed significantly between all concentrations of salt. The values of these parameters decreased as salinity levels increased. Germination index and seed stamina declined significantly with increasing salinity (Fig. 1). Dry weight of stems, total dry weight and root length declined with increasing salinity at all salt concentrations. Succulence at salinity of 0.1 mol·L^{-1} was greater than for control treatment of *A. albida* and *A. salicina* (Fig. 2, Table 3). Dry weight of stems decreased with increasing salinity, but was similar at 0.1 and 0.2 mol·L^{-1}. Different stages of seed germination are shown in Fig. 3.

Table 3. Effect of the interaction between different concentrations of salinity and species type on biomass

Index	*A. albida*				*A. salicina*			
	control	0.1 mol·L^{-1}	0.2 mol·L^{-1}	0.3 mol·L^{-1}	Control	0.1 mol·L^{-1}	0.2 mol·L^{-1}	0.3 mol·L^{-1}
Succulence (SU, mg)	88.3	88.67	88.33	.00	86.67	87.33	55.67	0.00
Stem length (SL, mm)	4.2^{bc}	2.31^{ab}	1.36^{ab}	.00^{a}	8^{d}	6.36^{cd}	0.3^{a}	0.00^{a}
Root length (RL, mm)	2.35	0.25	0.11	0.00	3.4	2.33	0.1	0.00
Total length (TL, mm)	6.55^{b}	2.56^{a}	1.48^{a}	0.00^{a}	11.14^{c}	8.7^{bc}	0.4^{a}	0.00^{a}
Fresh weight of stem (FWS, mg)	231.6^{e}	59.33^{bc}	49.67^{ab}	0.00^{a}	108.67^{cd}	125^{d}	6.33^{ab}	0.00^{a}
Fresh weight of root (FWR, mg)	28^{b}	3.67^{a}	2.33^{a}	0.00^{a}	9^{a}	15^{ab}	4.33^{a}	0.00^{a}
Dry weight of stem (DWS, mg)	26.67	8.67	5.67	0.00	14.33	14.67	1	0.00
Dry weight of root (DWR, mg)	7	1.33	1	0.00	2.67	1.33	0.33	0.00
Total fresh of weight (TFW, mg)	259.67^{d}	63^{ab}	52^{a}	0.00^{a}	117.67^{bc}	140^{c}	10.67^{a}	0.00^{a}
Total dry weight (TDW, mg)	33.67	10	6.67	0.00	17	16	1.33	.00

Notes: Means followed by different lower-case letters within columns are significantly different (p<0.01).

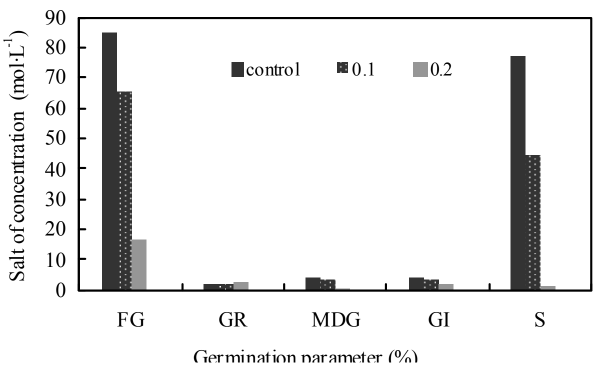

Fig. 1 Comparison of means of germination factor in different concentrations of salts (0.1, 0.2 mol·L^{-1}**) and control.** (FG: final germination (%), GR: germination rate (%), MDG: mean daily germination (%), GI: germination index (%), S: seed stamina (mm))

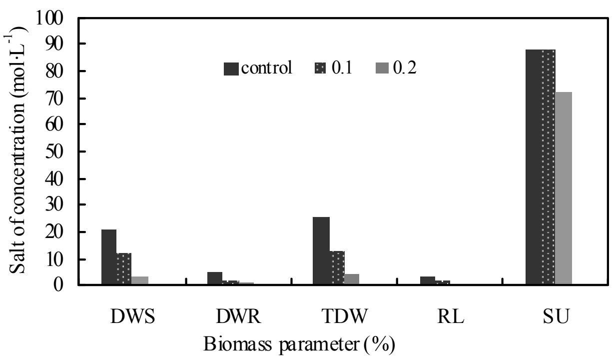

Fig. 2 Comparison of means of biomass factor in different concentrations of salts (0.1, 0.2 mol·L^{-1}**) and control.** (DWS is Dry weight of stem (mg), DWR is dry weight of root (mg), TDW is total dry weight (mg), RL: root length (mm), SU is succulence (mg)).

0.3 mol·L^{-1} 0.2 mol·L^{-1} 0.1 mol·L^{-1} 0.3 mol·L^{-1} 0.2 mol·L^{-1} 0.1 mol·L^{-1}

Fig. 3 Different stages of *Acacia salicina* and *Acacia albida* germination in salinity levels of control, 0.1 mol·L^{-1}, 0.2 mol·L^{-1}, 0.3 mol·L^{-1} NaH_2PO_4. H_20

Discussion

In plants, salt stresses involve complex and variable response mechanisms to ion transport and accumulation that relate to different metabolic activities of various organs. Salt stress is therefore, a significant factor in plant growth and tissue structure (Chelli-Chaabouni et al. 2010). Salt stress reduced germination and also delayed the emergence of seeds (Jamil et al. 2006). Similar results were obtained in this study.

Biomass production and germination of *A. salicina* and *A. albida* varied significantly by salinity level, species, and interaction between these factors. Biomass production and germination of the two species were similar at different salinity levels and these parameters decreased with increasing concentrations of salt (Tables 2 and 3) expect for fresh weight of root and total fresh weight in 0.1 $mol \cdot L^{-1}$concentration salt for *A. salicina*. These results are in agreement with findings of other investigators (Yokota 2003; Rehman et al. 1997). Fresh weight of stems of *A. albida* and control seedlings was higher than for other treatments and *A. salicina*, while maximum total length was recorded for *A. salicina*. This suggests that more accumulation of salt in *A. albida* caused increasing weight and prevented vertical growth. A similar observation was reported by Sarwar Khan et al. (2009), but with different species. They reported that maximum shoot and root length was recorded from *A. nilotica* against *A. ampliceps* at the same level of salinity (18 $dS \cdot m^{-1}$ ECe).

Fresh weight of stems was similar for the two species and the values of these parameters at all salinity levels were greater than the fresh weight of roots. This suggests that the weight of roots does not affect the total weight of plants, thus, stem weight was more strongly affected than of root weight at all salinity levels. The lengths of roots and stems are the most important parameters for salt stress because roots absorb water from the soil and stems supply water to the rest of the plant. In this study, maximum lengths of stems and roots were recorded for control and 0.1 $mol \cdot L^{-1}$concentration salt treatment of *A. salicina*. *A. salicina* was found to be superior to *A. albida* in its ability to emerge and survive under saline conditions.

Seedlings emerged and survived at 0.3-$mol \cdot L^{-1}NaH_2PO_4.H_20$ and the impact of increasing salt concentration on seed germination was less pronounced for *A. salicina* than for *A. albida* (Fig. 3). This supports the observations of Madsen and Mulliga (2006) for different species of *A. salicina* in comparison with *A. oucalyptus*. Final germination, seed stamina, germination index, total length and total root weight for both species decreased with increasing concentrations of salt (Tables 2 and 3). Similar results were reported by Jamil et al. (2006), who found that salt stress reduced seedling biomass (root and stem length, fresh and dry root and shoot weight) of *Dodonaea viscose*. Meloni et al. 2008 reported significant reduction in root and shoot growth of *Prosopis alba* with increasing concentration of NaCl. In control treatments and 0.1 $mol \cdot L^{-1}$ salt concentration, most seeds germinated for both of our study species and this could be a reason for the successful germination of these species in saline soil although few seeds germinated at the 0.2 and 0.3 $mol \cdot L^{-1}$salinities. At high salinity, the passage of salt to the seed interior is hindered by the seed cover. Not only salinity, nutrient availability, seed weight and light, but also interactions between these factors can control the germination of seeds (Atia et al. 2009).

Conclusion

Tolerance to salinity was greater for *A. salicina* than for *A. albida* and tolerance to salinity in the two species was low at 0.2 and 0.3 $mol \cdot L^{-1}$ as compared with the control. However, *A. salicina* and *A. albida* showed salt tolerance at germination and seedling growth stage at low salinity (0.1 $mol \cdot L^{-1}$). Increasing salinity reduced germination rates and final germination. This study clearly demonstrated that the germination of *A. salicina* and *A. albida* seeds was influenced not only by salt concentration, but also by species and interactions between species and salt concentration. Based on the results of this study, we recommend *A. salicina* for planting in saline soils.

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